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# Pesticide Assessment Guidelines, Subdivision F Revised Policy for Acute Toxicity Testing

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**16. Abstract (Limit: 200 words)**

In response to the need to improve scientific and regulatory decisions and to provide additional guidance for evaluating the acute toxicity of chemical exposures under the Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Control Act. This action builds upon a previous revision of the acute toxicity testing strategy to reduce the use of experimental animals while providing adequate information about chemical safety.

The Environmental Protection Agency is disseminating this notice to industry, governmental bodies, scientific societies, animal welfare groups and interested parties to apprise them of our new position. The Agency's acute toxicity testing guidelines are being revised to reflect the positions articulated in this policy.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

SEP 22 1988

Re: Revised Policy for Acute Toxicity Testing

Appended is a revised policy for evaluating the acute toxicity of chemical exposures under the Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Control Act. This action builds upon a previous revision of the acute toxicity testing strategy to reduce the use of experimental animals while providing adequate information about chemical safety.

The Environmental Protection Agency is disseminating this notice to industry, governmental bodies, scientific societies, animal welfare groups and interested parties to apprise them of our new position. The Agency's acute toxicity testing guidelines are being revised to reflect the positions articulated in this policy.

Victor J. Kimm
Acting Assistant Administrator
for Pesticides
and Toxic Substances

Enclosure

# Alternative Methodology for Acute Toxicity Testing

The Environmental Protection Agency announces a revision to its approach to acute toxicity testing in fulfillment of actions under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA). This revision reflects the Agency's concern about animal welfare and its continued efforts to reduce the impacts on animals of EPA's testing requirements. While maintaining the tiered approach adopted in 1984, the Agency now recommends (when appropriate) the use of abbreviated test methods and consideration of using only one sex, as a means of reducing the numbers of animals in deriving important information on acute toxicity.

## Background

EPA considers the evaluation of toxicity following short-term exposure to a chemical (i.e., acute toxicity) to be a limited but integral step in the assessment of the toxic potential of a chemical substance under the regulatory framework of its pesticide and toxic substances programs. The Agency also supports measures dedicated to reduce the use of animals in toxicity testing and conducts research on test methods which can lead to further reduction or elimination of animal usage and suffering. Through the careful selection of test methodology and maximization of the data obtained from acute studies, EPA strives to achieve a balance between the welfare of animals and the need to utilize animals in evaluating chemical safety.

The approach to acute toxicity testing previously given in EPA's Test Guidelines (U.S. Env. Prot. Agency, 1978; 1979) emphasized the determination of the median lethal dose (LD50) with a 95% confidence interval. A 1984 update of the guidelines,

published in 1985 (U.S. Env. Prot. Agency, 1985) stated that the Agency discouraged the uses of the "classical" LD50 test employing large numbers of animals for determination of lethality only. Instead, the Agency emphasized the use of a tiered approach to obtain acute toxicity data which reduced the number of animals used, but maximized the amount of relevant information that could be obtained from such testing. That approach included the following:

a. Using Data From Structurally Related Chemicals. The Agency encourages the review of existing acute toxicity information on chemical substances that are structurally related to the agent under investigation. Using this approach, one may be able to compile enough information from these surrogate chemicals to make preliminary safety evaluations that reduce the need for further animal testing or which indicate the type of testing to be pursued.

b. "Limit" Test. When information on structural analogs is inadequate, one should consider the "limit" test. The relative toxicity of a chemical is determined by professional judgement; for chemicals judged to be relatively non-toxic, a single group of animals is given a large dose of the agent. If no lethality is demonstrated, no further testing for this information is pursued.

c. Multifaceted Testing. A three-dose multiple endpoint evaluation may be important for those substances judged to be relatively toxic or which demonstrate lethality in the limit test. Using this procedure, animals are evaluated as to the onset, duration, intensity, and reversibility of behavioral effects, body weight changes and lethality; all animals are submitted to gross necropsy. Histopathology and certain follow-up studies may be warranted where there are gross indications of target organ toxicity.

Present Revision.

EPA has reevaluated its data needs on acute toxicity and continues to espouse the tiered approach that was developed in the 1984 update. Thus, the first consideration for a chemical for which there is no acute toxicity data, should be a review of structurally related compounds, followed by the limit test when appropriate. In those cases where testing beyond the limit test is indicated, consideration should be given to well-designed abbreviated test schemes which employ minimal numbers of animals, as discussed below. In most cases, it is expected that these tests can be structured to give enough information on acute toxicity to obviate the need for further acute studies (e.g., the three-dose multi-faceted testing approach). We continue to stress the need for collecting information on behavioral effects, gross pathology and lethality (as developed in "c" above).

While more complete animal testing may be necessary in some cases (based on scientific evidence from the abbreviated test, e.g., delayed toxicity, unusual central nervous system effects, irreversible effects), the Agency generally supports limiting such tests to those using the lowest feasible number of animals.

Several abbreviated methods to investigate acute toxicity have been developed over the years. Some of them have rather extensive data bases and have been validated against more traditional test methods which estimate median lethal dose. Their merit lies in the fact that they allow for the evaluation of the full spectrum of acute responses; numerical calculations can be made; and fewer animals may be employed in the generation of the information than with most other approaches. For some methods, statistical calculations are simple or are aided by tables.

EPA has investigated four methodologies that might be used. These include (1) the approximate lethal dose method[1] of Deichmann and Le Blanc (1943); (2) the moving averages method[2] of Thompson (1947); (3) the up-and-down method[3] of Dixon and Mood (1948) and Dixon (1965); and (4) the cumulant method of Reed-Muench[4] (1938).

The methods vary as to the assumptions that are made, the number of groups of animals and number of animals per group. Toxicologists should be familiar with these differences before employing a given method. For instance, the up-and-down method is especially difficult to apply when chemicals induce delayed toxic effects. Therefore, other methodologies may be more appropriate. When an alternative method for acute toxicity testing is selected, a rationale for such a selection should accompany the submission. The Agency solicits discussions with data generators on still other methods that may be employed.

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[1]The approximate lethal dose method was further refined by Deichmann and Mergard (1948); these authors performed eighty-seven determinations (calculated by the methods of Behrens (1929) and Bliss (1938)). The approximate lethal dose method was also used by Kennedy et al. (1986).

[2]The moving averages method was refined by Weil (1952, 1983) and Gad and Weil (1982), and was used by Smythe and Carpenter (1944, 1948) and Smythe et al. (1949, 1951, 1954, 1962).

[3]The up-and-down method was recently used and refined by Bruce (1985, 1987) (calculated by the method of Bliss (1938)). The up-and-down method was also used by Brownlee et al. (1953), Dixon and Massey (1957), Klassen and Plaa (1967) and Hsi (1969).

[4]The Department of Defense has had considerable experience using the Reed-Muench method with a large number of chemicals (F. Vocci, personal communication); it has also been used by Lorenz and Bogel (1973), Bhan (1974), Aubert and Amdral (1979), and Thakur and Fezio (1981).

The Agency emphasizes that parallel assays on male and female animals to determine an approximate estimate of acute toxicity need not be routinely determined, since male and female animals of the same strain generally show only slight and insignificant differences in susceptibility to toxic agents. However, for some chemicals, one sex may be somewhat more sensitive than the other (Muller and Kley (1982); Schutz and Fuchs (1982); (Bruce (1985)). Cassarett and Doull (1980) indicate that the class of compound is important in specific sex differences. De Pass et al. (1984) showed that for 91 chemicals tested for oral toxicity in rats, females were slightly more sensitive than males ($p<.001$). Muller and Kley (1982) performed 152 parallel studies on male and female animals for which 129 showed no significant differences. However, when statistically significant differences were observed (23 compounds), 17 were more toxic to females. Therefore, consideration should be given to limiting studies to the more sensitive sex. Previous history on the class of chemical being evaluated would be helpful in making this determination. For confirmation, a few animals of the other sex should also be tested.

In summary, EPA has modified its approach to acute toxicity testing, recognizing that appropriate information for safety evaluation can be developed using fewer animals than had been recommended in the past. We strongly urge industry to use these abbreviated test methodologies, whenever appropriate, as replacements for the three-dose multifaceted method EPA previously had recommended. Four such methodologies which might be used have been identified; other methods may also be employed, if adequate rationale can be provided. It is expected that studies will still include behavioral observations, gross necropsy and ancillary observations, as before.

EPA urges industry to begin submitting data obtained with alternate methods which use fewer animals on a routine basis; the Agency is planning to revise its testing guidelines to incorporate the above guidance. We plan to accept only newly generated industry data that conforms with our revised guidance unless an adequate rationale (e.g., data generated in accordance with regulatory requirements other than those of EPA) accompanies the submission; data without a rationale may be returned to the submitter.

The Agency encourages the public to comment on this position and provide information on still other alternate methodologies which have progressed to a stage of validation which would be acceptable to the scientific community.

## References

Aubert M.F.A. and Amdral L. (1979). Potency Testing of Veterinary Vaccines Containing Inactivated Virus. Comp. Immunol Microbiol. Infect. Dis., 1, 341-349.

Behrens B. (1929). The Assay of Digitalis on Frogs. Arch. Exptl. Path. Pharmakol., 140, 237-256.

Bhan A.K. (1974). Computing LD50 More Efficiently and Accurately - A Modification of Reed-Muench Method. Indian Soc. Nucl. Agricul. Biol., 3, 5-6.

Bliss C.I. (1938). Determination of the Small Dosage Mortality Curve from Small Numbers. Quart. J. Year Book Pharm., 11, 192-216.

Brownlee K.A., Hodges J.L. and Rosenblatt M. (1953). The Up-and-Down Method With Small Samples. Amer. Statist. Assoc. J., 48, 262-277.

Bruce R.D. (1985). An Up-and-Down Procedure for Acute Toxicity Testing. Fundam. Appl. Toxicol., 5, 151-157.

Bruce R.D. (1987). A Confirmatory Study of the Up-and-Down Method for Acute Oral Toxicity Testing. Fundam. Appl. Toxicol., 8, 97-100.

Casarett L.J. and Doull J. (1980). Factors Influencing Toxicology. In: Toxicology, The Basic Science of Poisons, J. Doull, C.D. Klaasen and M.O. Amdur, eds., 77-88. New York: Macmillan Publishing Co., Inc.

Deichmann W.B. and LeBlanc T.L. (1943). Determination of the Approximate Lethal Dose With About Six Animals. J. Indust. Hyg. Toxicol., 25, 415-417.

Deichmann W.B. and Mergard E.G. (1948). Comparative Evaluation of Methods Employed to Express the Degree of Toxicity of a Compound. J. Indust. Hyg. Toxicol., 30, 373-378.

De Pass L.R., Myers R.C., Weaver E.V. and Weil C.S. (1984). An Assessment of the Importance of Number of Dosage Levels, Number of Animals per Dosage Level, Sex and Method of LD50 and Slope Calculation in Acute Toxicity Studies. In: Acute Toxicity Testing: Alternative Approaches, A.M. Goldberg, ed. (Alternative Methods in Toxicology, Vol. 2), 142-153. New York: Mary A. Liebert, Inc., Publisher.

References (contd.)

Dixon W.J. (1965). The Up-and-Down Method for Small Samples. Amer. Statist. Assoc. J., 60, 967-978.

Dixon W.J. and Massey F.J. Jr. (1957). Sensitivity Experiments, In: Introduction to Statistical Analysis, 318-327. New York: McGraw-Hill.

Dixon W.J. and Mood A.M. (1948). A Method for Obtaining and Analyzing Sensitivity Data. Amer. Statist. Assoc. J., 43, 109-126.

Gad S.C. and Weil C.S. (1982). Statistics for Toxicologists. In: Methods in Toxicology, A.W. Hays, ed., 273-309. New York: Raven Press.

Hsi, B.P. (1969). The Multiple Sample Up-and-Down Method in Bioassay. Amer. Statist. Assoc. J., 64, 147-162.

Kennedy, G.L. Jr., Ferenz, R.L. and Burgess, B.A. (1986). Estimation of Acute Oral Toxicity in Rats by Determination of the Approximate Lethal Dose Rather Than the LD50. J. Appl. Toxicol. 6, 145-148.

Klassen, C.D. and Plaa, G.L. (1967). Relative Effects of Various Chlorinated Hydrocarbons on Liver and Kidney Function in Dogs. Toxicol. Appl. Pharmacol., 10, 119-131.

Lorenz R.J. and Bogel K. (1973). Methods of Calculation. Method of Reed and Muench. Laboratory Techniques in Rabies. WHO Monograph Series 23, 3rd Ed., 329-335.

Muller H. and Kley H.P. (1982). Retrospective Study on the Reliability of an "Approximate LD50"[5] Determined with a Small Number of Animals. Arch. Toxicol., 51, 189-196.

Reed L.J. and Muench H. (1938). A Simple Method for Estimating Fifty Percent Endpoints. Amer. J. Indust. Hyg. Toxicol., 30, 373-378.

Schutz E. and Fuchs H. (1982). A New Approach to Minimizing the Number of Animals Used in Acute Toxicity Testing and Optimizing the Information of Test Results. Arch. Toxicol., 51, 197-220.

Smythe H.F. Jr. and Carpenter C.P. (1944). Place of the Range-Finding Test in the Industrial Toxicology Laboratory. J. Indust. Hyg. Toxicol., 26, 269.

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[5] "Approximate LD50" in the title of this paper is placed in quotation marks so as not to confuse it with the method of Deichman and Le Blanc (1943).

References (contd.)

Smythe H.F. Jr. and Carpenter C.P. (1948). Further Experience with the Range-Finding Test in the Industrial Toxicology Laboratory. J. Indust. Hyg. Toxicol., 30, 63.

Smythe H.F. Jr., Carpenter C.P. and Weil C.S. (1949). Range-Finding Toxicity Data: List III. J. Indust. Hyg. Toxicol., 31, 60.

Smythe H.F. Jr., Carpenter C.P. and Weil C.S. (1951). Range-Finding Toxicity Data: List IV. AMA Arch. Indust. Hyg. Occ. Med., 4, 119.

Smythe H.F. Jr., Carpenter C.P., Weil C.S. and Pozzani U.C. (1954). Range-Finding Toxicity Data: List V. AMA Arch. Indust. Hyg. Occ. Med. 10, 61.

Smythe H.F. Jr., Carpenter C.P., Weil C.S., Pozzani U.C., and Striegel J.A. (1962). Range-Finding Toxicity Data: List VI. Amer. Indust. Hyg. Assoc. J., 23, 95.

Thakur A.K. and Fezio W.L. (1981). A Computer Program for Estimating LD50 and It's Confidence Limits Using Modified Behrens-Reed-Muench Cumulant Method. Drug Chem. Toxicol., 4, 297-305.

Thompson, W.R. (1947). Use of Moving Averages and Interpolation to Estimate Median Effective Dose. Bacteriol. Rev. 11, 115-145.

U.S. Environmental Protection Agency. (1978). Proposed Guidelines for Registering Pesticides in the U.S. Hazard Evaluation: Humans and Domestic Animals. Fed. Reg. 43, 37336-37345; 37351-37356. (August 22).

U.S. Environmental Protection Agency. (1979). Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules and Proposed Good Laboratory Practice Standards for Health Effects. Fed. Reg. 44, 44054-44059; 44066-44067. (July 26).

U.S. Environmental Protection Agency. (1985). Health Effects Testing Guidelines. Fed. Reg. 50, 39397-39398. (September 27)

Vocci. F. (1988). Personal Communication (Unpublished Data).

Weil C.S. (1952). Tables for Convenient Calculation of Median Effective Dose (LD50 or ED50) and Instructions on Their Use. Biometrics 8, 249-263.

Weil C.S. (1983). Economical LD50 and Slope Determinations. Drug Chem. Toxicol. 6, 595-603.